REMARKS

Claims 25-47 are currently pending in this application. Claims 25 and 26 are currently in independent form. Claims 25, 29-30, 45-46 have been withdrawn from consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention. Claim 47 is newly added, support for which can be found, for example in paragraph [0062] of the specification as filed. Claims 26-28, 31-44, and 47 are under examination with regard to SEQ ID NOS. 1-2.

Kamerbeek in view of Furugen, Hogan and Buck

Claims 26-28 and 31-41 are rejected under 35 U.S.C. §103(a) as being obvious over Kamerbeek et al. (J Clin Microbiol, 1997 vol. 35, pp. 907-914) in view of Furugen (Microbial Pathogenesis 2001 vol. 30, pp 129-138), United States Patent No. 5,541,308 to Hogan et al. and Buck et al (Biotechniques (1999) 27(3):528-536) for the reasons indicated on pages 4-8 of the Office Action. Applicants respectfully traverse this rejection.

The invention of claim 26 is directed to a method for differentiating Mycobacterium species based on target gene encoding for histone like proteins such as *hupβ*. The method of claim 26 includes obtaining DNA from culture or from clinical samples; amplifying a part of the target gene encoding for histone like proteins such as *hupβ* of Mycobacterium species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers of SEQ. ID No. 1 and SEQ. ID No. 2; and detecting said amplified fragment of the hupβ gene to detect the presence of Mycobacterial species or not and differentiating Mycobacterium tuberculosis from Mycobacterium bovis based on the size of the amplified fragment.

The Examiner applies Kamberbeek to teach the simultaneous detection and differentiation of *M. tuberculosis* amplification of a DR region with known interspersed space repeats by PCR. The method of Kamerbeek (e.g. spoligotyping) is focused on the examination and analysis of a Direct Repeat Region (DR Region) in the DNA. The DR Region is not located near the *hupβ* gene in the DNA. Specifically, the DR Region commences from 31, 19, 276 base pair (bp) and stretches up to 31, 23, 484bp; whereas the *hupβ* gene of *M. tuberculosis (Rv2986c)* commences from the 33, 43, 176 base pair and extends up to 33, 43, 820 and the homologue in

M. bovis is located between bp 32, 99, 736 and 33, 00, 380. As it can be seen, the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis* are located more than 22,000 bp down stream from the DR Region. Therefore, because the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis* are located more than 22,000 bp downstream from the DR Region, it would not have been obvious to one of ordinary skill in the art to assume that similar conclusions and differentiations could be made about the DR Region and the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis*.

Additionally, one of ordinary skill in the art would not have known to utilize the size differences in the *hupβ* gene and its homologue in *M. bovis* by examining the DR Region. Particularly, the identification of the differences in gene size of the *hupβ* gene of *M. tuberculosis (Rv2986c)* and its homologue in *M. bovis (Mbo3010c)* provided for the specific design of the primers (SEQ. ID No. 1 and SEQ. ID No. 2). The design of the specific primers used for the differentiation of Mycobacterium species as taught by claim 26, arose through the analysis of the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis*. The design of primers specific to the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis*, would not have been obvious through the analysis of the upstream genome related to the DR Region.

Therefore, because the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis* are not located near the DR Region taught by Kamerbeek and the analysis of the DR Region would not provide the methods including the primers of the claimed invention used for differentiation based on the *hupβ* gene of *M. tuberculosis* and its homologue in *M. bovis*, claim 26 is not obvious in view of Kamerbeek.

Additionally the targets utilized by the method of the claimed invention are realistically distinct from the targets of Kamerbeek. The claimed N-PCR technique for differential identification of *M. tuberculosis* and *M. bovis* in culture and directly in clinical specimens is not taught or suggested by the regions targeted by Kamerbeek et al.

Furugen is applied to teach the presence of a DNA binding motif in homologous proteins derived from *M. tuberculosis* and other mycobacterial species, despite variation in size and amino acid content. Particularly, Furugen relates to the function of the DNA binding protein (MDP1) derived from *M. bovis*. The comparative amino acid sequence analysis described by

Furugen shows DNA binding proteins, with variation in amino acid content and number. However, Furugen does not teach or suggest the claimed differentiation and identification of mycobacterial species. Particularly, one of ordinary skill in the art reviewing Furugen would focus on the protein not the exploitation of the differences between the closely related mycobacterial species M. *tuberculosis* and M. *bovis*.

Additionally, Furugen teaches that there is a 9 amino acid difference between *M. bovis* and *M. tuberculosis*. However, Applicants assert that it is not obvious from the teachings of Furugen that this difference can be used for differentiating these two closely genetically and anti-genetically related mycobacterial pathogens, neither at the protein nor at the nucleotide level. Further, it was the knowledge acquired by Applicants which demonstrated for the first time that the 27 nucleotide difference could be exploited for differential identification of *M. tuberculosis* and *M. bovis* in culture and directly in human and bovine.

Merely being aware that 9 amino acids are present in the *hupβ* gene of *M. tuberculosis* is not complete information; due to degeneracy of the genetic code and codon bias, it is important to determine the actual nucleotide sequence. Only after obtaining accurate and indisputable information were the primers required for targeting the *hupβ* gene designed. The design of primers is determined by set guidelines and norms, which are universal for all primers designed for PCR, probes, etc. Even though sequence software programs are useful tools for employing broad principles, they are not adequate in practice, as each primer is required to be modified to improve on the primer sequence and, hence, the efficacy of the primers in terms of target specificity. Owing to inherent peculiarities and nuances of sequences, PCR programs, etc., need to be tailored for each pair of primers for PCR based amplification of the target gene, hence what has been described by Hogan (i.e., the use of specific primers), and Buck (i.e., the equivalence of primers), is a strategy and does not provide the conclusiveness that is required for primer design of identified gene targets. Therefore, despite the public information on primer design, this knowledge information is insufficient and would not teach or suggest the claimed method. It is the additional inputs, tempered by actual practice that proves the utility of the primers designed for the described application.

For at least the aforementioned reasons, none of Kamerbeek, Furugen, Hogan or Buck provides any reason to believe that the method of differentiation based on the DR Region would teach or suggest the claimed primers (SEQ. ID No. 1 and SEQ. ID No. 2) and probes (SEQ. ID No. 7) used to differentiate mycobacterium species based on target gene encoding for histone like proteins. Therefore, Kamerbeek, in view of Furugen, Hogan and Buck does not teach, or suggest each and every element of claims 26 and, therefore, claim 26 is not obvious over the teachings of Kamerbeek in view of Furugen, Hogan and Buck .

Claims 27-28 and 31-41 depend from and further limit claim 26 and are believed to be patentable for at least the aforementioned reasons. Withdrawal of the rejections under 35 U.S.C § 103(a) and allowance of claims 26-28 and 31-41 are respectfully requested.

Kamerbeek in view of Furugen, Hogan and Buck, Taylor and Cuende

Claims 42-44 are rejected under 35 U.S.C. §103 (a) as being obvious over Kamerbeek et al. in view of Furugen, Hogan et al. and Buck et al. as applied to claims 26-28 and 31-41 and further in view of Taylor (J. Clin. Microbiol. 1997, vol. 35 pp. 79-85) and Cuende et al. (Med Clin (Barc) 1995, vol. 104, pp. 207-210) (abstract only) for the reasons set forth on pages 8-10 of the Office Action.

Claims 42-44 depend directly or indirectly from and further limit claim 26. Taylor is applied to teach PCR-restriction fragment length polymorphism to identify 28 species of clinically encountered mycobacteria for identification.

Taylor describes a PCR-RFLP technique for identification of a range of mycobacterial species and to differentiate *Mtb* complex from other rapidly growing mycobacteria. Herein lies the difference between the techniques. The 2 step N-PCR technique described in the patent application is poles apart from the PCR -RFLP technique described by Taylor et al. Taylor's technique does not attempt to identify members of the *Mtb* complex, whereas the N-PCR technique using the *hupβ* gene as a target does precisely that. The PCR-RFLP described in the application is adjunct information to stress and confirm the basis of the usage of the *hupβ* gene of differentiating the two pathogenic species, namely, *M. tuberculosis* and *M. bovis*.

Cuende is applied to teach amplification followed by RFLP analysis using HpaII and analysis on gel electrophoresis to generate 13 different patterns to type clinical isolated mycobacteria.

However, neither Taylor nor Cuende account for the deficiencies of the teachings of Kamerbeek, Furugen, Hogan and Buck. Particularly, neither Taylor nor Cuende provide any reason to believe that the method of differentiation based on the DR Region would teach or suggest the claimed primers (SEQ. ID No. 1 and SEQ. ID No. 2) and probes (SEQ. ID No. 7) used to differentiate mycobacterium species based on target gene encoding for histone like proteins. Therefore, Kamerbeek in view of Furugen, Hogan, Buck, Taylor and Cuende does not teach or suggest each and every element of claims 42-44 and, therefore, claims 42-44 are not obvious over the teachings of Kamerbeek, Furugen, Hogan and Buck. Withdrawal of the rejections under 35 U.S.C §103 (a) and allowance of claims 42-44 are respectfully requested.

CONCLUSION

In view of the foregoing remarks, it is respectfully submitted that all pending claims are allowable. Accordingly, reconsideration of the rejections and allowance of claims 26-28, 31-44, and 47 are respectfully requested.

Respectfully submitted,

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